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Studies on pentosans in Indian wheat (*Triticum aestivum*) varieties in relation to chapati making quality

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Abstract

Different wheat varieties were screened for chapati making characteristics, of which DWR-162 and GW-322 showed good and MACS-2496 and HD-2189 exhibited poor chapati making quality. Various polysaccharide fractions such as water-soluble, barium hydroxide-soluble and alkali-soluble polysaccharides (viz., hemicelluloses A and B) were isolated from these wheat varieties and their carbohydrate composition was analysed by gas liquid chromatography. Glucose was the major sugar, along with small amounts of arabinose and xylose in water-soluble fractions. Barium hydroxide extract mainly consisted of arabinose and xylose, along with small amounts of glucose. Hemicellulose A fractions contained mainly xylose. Arabinose, xylose and glucose were the major sugars observed in hemicellulose B fractions. Hence, hemicellulose A may be more xylan type, while, hemicellulose B appears to be of complex hemicellulosic nature. Alkali-insoluble residue was basically cellulosic in nature and was strongly associated with arabinoxylans. The studies indicated that arabinose to xylose (A/X) ratio was higher in varieties having good chapati making quality compared to poor varieties in the flour and barium hydroxide extract, which are rich in arabinoxylan type polysaccharides. The ratio of xylose to glucose (X/G) was higher in good chapati making varieties compared to poor varieties.

Keywords: Wheat; Chapati; Pentosans; Arabinoxylans; Polysaccharides

1. Introduction

Wheat is a major cereal in India and is consumed mainly in the form of unleavened flat bread known as chapati (Prabhasankar, Manohar, & Gowda, 2002). Chapati is usually prepared from whole-wheat flour and the desired quality parameters in chapati are greater pliability, soft texture, light creamish brown colour, slight chewiness and baked wheat aroma (Haridas Rao, Leelavathi, & Shurpalekar, 1986). Apart from starch and proteins, wheat flour contains pentosans in minor amounts which are located in the cell walls of wheat grain (Fincher & Stone, 1986). Pentosans (Arabinoxylans) are the major non-starch polysaccharides in wheat and consist of xylan backbone with branches of arabinose residues in various linkages. The pentosans are known to play an important role in the water balance of dough (Courtin & Delcour, 2002), rheological properties of dough (Michniewicz, Biliaderis, & Bushuk, 1991), retrogradation of starch (Gudmundsson, Eliasson, Bengtsson, & Aman, 1991) and bread making quality (Delcour, Vanhamel, & Hoseney, 1991; Shogren, Hashimoto, & Pomeranz, 1987). These arabinoxylans compete with other constituents of dough for water added to the flour (Izydorczyk & Biliaderis, 1995). Oxidative gelation has also been shown to increase the hydration quality of arabinoxylans (Izydorczyk, Biliaderis, & Bushuk, 1990). The ability of arabinoxylans to imbibe and hold water was found to increase with the cross linking density of the gel network (Izydorczyk & Biliaderis, 1995). The arabinose to xylose ratio is a direct measure for the degree of substitution and is therefore an important structural characteristic of arabinoxylans (Courtin & Delcour, 1998). The ratio of arabinose to xylose in different varieties of wheat has been shown to vary from 0.5 to 1.0 (Izydorczyk

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& Biliaderis, 1995). Pentosans of different wheat flours have been compared by several workers (Delcour, Van win, & Grobet, 1999; Izydorczyk & Biliaderis, 1995). Lineback, Kakuada, and Tsen (1977) found differences in the carbohydrate composition of pentosans isolated from different wheat flours. The chemical composition and structures of arabinoxylans vary significantly in different cereals and among wheat varieties and has been a subject of great academic interest (Aspinall, 1980). Variation in the amount, chemical composition and structures of pentosans brings about differences in their physico-chemical, solubility and functional properties. Pentosans in wheat are known to be best extracted with barium hydroxide (Gruppen, Hamer, & Voragen, 1991; Nandini & Salimath, 2003). Alkali-soluble pentosans (hemicellulose A and B) have also generated much interest (Salimath & Tharanathan, 1982). Alkaline hydrogen peroxide has been used for the extraction of arabinoxylans from wheat bran (Maes & Delcour, 2001). There have been few studies to relate these pentosans with respect to chapati making quality. Hence, the present study was undertaken to isolate pentosan rich fractions from different Indian wheat varieties and determine their chemical composition and to assess their implications for intervarietal differences in chapati making quality.

2. Materials and methods

2.1. Materials

DWR-162 variety of wheat (*Triticum aestivum* L.) was obtained from University of Agricultural Sciences, Dharwad and MACS-2496, GW-322, HD-2189 varieties were procured from Agharkar Research Institute, Pune, India. Cleaned wheat was milled into whole flour (100% extraction) in a commercial disc mill. The flour obtained was cooled and passed through a 480 μm sieve for homogenization. Termamyl (EC 3.21.1. from *Bacillus licheniformis*) was procured from Novo Nordisk, Denmark. Glucoamylase and dialysis bags (Cellulose membranes, 12,000 MW cut off) were procured from Sigma, St. Louis, USA. All other chemicals and reagents used were of analytical reagent grade.

2.2. Methods

2.2.1. Chapati making quality

Chapatis were prepared and sensory properties were evaluated according to the method of Haridas Rao et al. (1986). Chapatis were evaluated by a trained panel of eight judges for the following parameters; appearance, tearing strength, pliability, taste and aroma and chewiness, using a 10-point scale. The chapatis having higher scores were considered to have better quality.

2.2.2. Extraction of polysaccharides

Various polysaccharide fractions from whole-wheat flour of different wheat varieties that varied in chapati making quality were isolated by employing methods as described earlier (Nandini & Salimath, 2001). The flour was treated with 70% alcohol to remove free sugars. The residue was cooked with water to gelatinize starch and then subjected to glucoamylase digestion. Water-soluble polysaccharides were isolated from the destarchified flour. The water-insoluble residue was used to extract barium hydroxide-soluble polysaccharide (Gruppen et al., 1991). The residue was then taken for the extraction of hemicelluloses (hemicellulose A and B) using 10% NaOH under nitrogen atmosphere. The extract was acidified with acetic acid (50%) in ice-cold temperature to pH 4.5 to obtain a precipitate (hemicellulose A) and the supernatant was dialyzed and lyophilized (hemicellulose B). The alkali-insoluble residue was dried by solvent exchange after removing salts.

2.2.3. Analytical methods

Estimation of total sugars was done by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and uronic acids by carbazole method (Dische, 1947). Sugar composition in the flour and water-insoluble fractions were analysed after solubilization with 72% sulphuric acid (in ice-cold temperature) followed by hydrolysis in 10% sulphuric acid at boiling water bath temperature for 6–8 h. The water-soluble fractions were hydolysed with 2 N trifluroacetic acid in sealed tubes at 100 °C for 5–6 h. The sugars were analysed by gas liquid chromatography as alditol acetates (Sawardekar, Slonekar, & Jeanes, 1967) on an OV-225 column at column temperature of 200 °C using a Shimadzu GLC.

3. Results and discussion

3.1. Chapati making quality

Sensory evaluation scores of chapatis prepared from four wheat varieties are given in Table 1. Chapatis prepared from DWR-162 and GW-322 had appealing light

Table 1
Sensory evaluation of chapati from different wheat varieties[a]

Wheat varieties	Appearance (10)	Tearing strength (10)	Pliability (10)	Taste and aroma (10)	Chewiness (10)	Overall quality (50)
DWR-162	8.8	8.3	8.3	8.3	8.3	42.0
GW-322[a]	8.2	8.6	8.6	8.3	8.0	41.7
MACS-2496[a]	7.3	7.4	7.2	6.2	7.1	35.2
HD-2189[a]	7.3	7.7	7.6	6.5	6.9	36.0

[a] Hemalatha et al. (in press).

brown colour. On the other hand MACS-2496 had relatively dark colour. Tearing strength data indicated that GW-322 followed by DWR-162 had desirable tearing strength. Chapatis made from GW-322 and DWR-162 were highly pliable. Highest sensory scores for taste and aroma were recorded for GW-322 and DWR-162. MACS-2496 and HD-2189 recorded significantly lower scores for taste due to bland taste. Chewiness properties showed that DWR-162 and GW-322 had higher scores. These chapatis were neither tough nor hard to chew and had optimum chewing properties. As shown by high overall quality scores, varieties DWR-162 and GW-322 yielded highly acceptable chapatis. MACS-2496 and HD-2189 yielded lower overall quality scores (Hemalatha, Manu, Bhagwat, Leelavathi, & Prasada Rao, in press).

3.2. Carbohydrate composition of DWR-162

Chapati, the flat unleavened baked product prepared from whole-wheat flour, is the main traditional staple food in the Indian sub-continent (Srivastava, Meyer, Haridas Rao, & Seibel, 2002). DWR-162 wheat variety revealed good chapati making characteristics (Table 1). Carbohydrate profile of DWR-162 variety of wheat flour and its isolated fractions are given in Table 2. Wheat flour and its various polysaccharide fractions were rich in carbohydrates and uronic acid content ranged from 2.6% to 5.7%. Sugar analysis of the flour indicated glucose, predominantly, with small amounts of arabinose and xylose and minor quantities of rhamnose/fucose and mannose. The ratio of arabinose to xylose in the flour was 1.26. Water-soluble polysaccharides were rich in glucose, which could arise from β-glucans, resistant starch and unhydrolysed starch molecules. Arabinose, xylose, rhamnose/fucose and mannose were also observed in minor amounts. Polysaccharides extracted with barium hydroxide were found to be rich in pentosans (78%) and contained a moderate amount of glucose (22%). This polysaccharide could be a complex mixture of arabinoxylans and β-glucans. Presence of β-glucan in arabinoxylan fractions has been reported earlier (Cleemput, Roels, Vanoort, Grobet, & Delcour, 1993). The ratio of arabinose to xylose in the barium hydroxide extract was 1.68, indicating that it might be due to higher degree of branching in these pentosans. Higher degree of branching was reported in some Canadian wheat flours of variable bread making quality (Izydorczyk & Biliaderis, 1995). Hemicellulose A contained xylose predominantly (66%), with small amounts of arabinose and glucose. This fraction may contain mainly xylan-type of polysaccharides and ratio of arabinose to xylose was low (0.14). High amounts of arabinose, xylose and glucose were observed in Hemicellulose B. Presence of glucose in this fraction may be due to the presence of β-glucans and resistant starch and unhydrolysed starch molecules. The alkali-insoluble residue still contained moderate amounts of arabinose and xylose. This suggests strong association of arabinoxylans with cellulose. Strong association of cellulose with non-starch polysaccharides was also reported in redgram (Swamy, Ramakrishnaiah, Kurien, & Salimath, 1991) and wheat (Nandini & Salimath, 2001).

3.3. Carbohydrate composition of GW-322

Wheat variety GW-322 that exhibited a good chapati making quality was used for the study. Carbohydrate composition revealed that this flour and its various polysaccharide fractions were rich in carbohydrate content (Table 3) and uronic acid content ranged from 2.1% to 6.7%. Glucose was the major sugar and small amount of arabinose and xylose with minor quantity of mannose and rhamnose/fucose were present in the flour. The ratio of arabinose to xylose was found to be 1.32 in whole-wheat flour. The water-soluble polysaccharide was rich in glucose (82%) and contained a small amount of arabinose and xylose. Water-soluble extracts are known to contain high amounts of β-glucans with associated pentosans. This could also include resistant starch and unhydrolysed starch formed during the extraction. The barium hydroxide extract was rich in arabinose, xylose (83%) and contained small amounts of glucose (14%). The ratio of arabinose to xylose was 1.14:1, indicating that it may be due to higher degree of branching in these pentosans. Izydorczyk and Biliaderis (1995) have reported A/X ratio ranging from 1.1 to 1.2 in Canadian wheat varieties. Large variations in the degree of branching of pentosans were also found among European wheat flours of different bread making

Table 2
Carbohydrate composition (%) of whole-wheat flour (DWR-162) and its isolated fractions[a]

Fractions	Yield	Total sugar	Uronic acid	Sugars identified (%)						
				Rha/Fuc	Ara	Xyl	Man	Gal	Glc	Ara/Xyl ratio
Flour	(100)	75.6	5.7	1.10	7.30	5.75	2.96	–	82.78	1.26
WSP	11.5	87.5	3.4	0.74	1.97	1.26	2.47	–	93.5	1.56
BE	3.6	78.1	3.0	–	49.0	29.0	–	–	22.0	1.68
Hem A	1.0	88.1	2.6	1.72	9.87	66.49	1.26	–	20.62	0.14
Hem B	1.0	80.0	2.8	–	26.66	38.87	2.48	–	31.97	0.68
AIR	3.5	73.4	3.6	–	29.20	17.55	2.07	–	51.15	1.66

[a] WSP, water-soluble polysaccharides; Hem A, hemicellulose A; AIR, alkali-insoluble residue; Fuc, fucose; Xyl, xylose; Gal, galactose; BE, barium hydroxide extract; Hem B, Hemicellulose B; Rha, rhamnose; Ara, arabinose; Man, mannose; Glc, glucose.

Table 3
Carbohydrate composition (%) of whole-wheat flour (GW-322) and its isolated fractions

Fractions	Yield	Total sugar	Uronic acid	Sugars identified (%)						
				Rha/Fuc	Ara	Xyl	Man	Gal	Glc	Ara/Xyl ratio
Flour	(100)	76.3	3.5	0.44	5.56	4.20	0.80	–	89.0	1.32
WSP	12.2	83.0	4.7	0.46	6.98	9.18	1.4	–	81.9	0.76
BE	4.2	80.2	2.1	–	44.5	38.9	–	2.90	13.7	1.14
Hem A	0.3	88.0	6.7	–	4.36	79.3	–	–	16.4	0.05
Hem B	0.5	90.0	4.5	–	13.5	62.9	–	–	23.6	0.21
AIR	3.2	74.0	3.8	–	15.6	11.8	2.20	–	70.4	1.32

Abbreviations as in Table 2.

quality (Cleemput et al., 1993). Both hemicelluloses A (79%) and B (63%) were rich in xylose, with small amounts of arabinose and glucose and a small amount of uronic acid was also present. These hemicellulosic fractions (hemicellulose A and B) may contain a mixture of xylan-type polysaccharide with small amounts of arabinoxylan type of polysaccharides. The associated glucose might originate from β-glucans and resistant starch formed during gelatinisation of starch. The alkali-insoluble residue was mainly cellulosic in nature with small amounts of associated arabinoxylans.

3.4. Carbohydrate composition of MACS-2496

The wheat variety MACS-2496 exhibited poor chapati making quality and the carbohydrate composition of the flour and its fractions are shown in Table 4. Wheat flour was rich in carbohydrate (70.8%) and the uronic acid content was 4.7%. Glucose was the major sugar and small amounts of arabinose and xylose were observed in the flour. The water-soluble polysaccharides contained glucose, mainly, with small amounts of arabinose and xylose and minor quantities of rhamnose and mannose. The high amount of glucose (87%) may be due to the presence of β-glucan type polysaccharide and resistant starch along with unhydrolysed starch molecules. β-Glucan content in wheat is reported to be 0.8% on a dry weight basis (Henry, 1987). The barium hydroxide extracted arabinoxylans (93.2%) contained principally, arabinose and xylose in the ratio of 0.8:1, indicating lower degree of branching. The ratio of arabinose to xylose in wheat endosperm may vary from 0.5 to 0.7 (Cleemput et al., 1993) but it is lower than wheat bran (Brillouet & Mercier, 1981). The presence of a small amount of glucose may be due to the resistant starch that may be formed during gelatinization of starch. The alkali-soluble polysaccharides were rich in carbohydrates. High amounts of xylose (43%) and glucose with small amount of arabinose and uronic acids were observed in hemicellulose A. This fraction may contain xylan-type polysaccharides along with small amounts of arabinoxylans and β-glucan type polysaccharide. Hemicellulose B was rich in pentosans (69%) and had a moderate amount of glucose. Alkali-insoluble residue still contained considerable amounts of (24%) arabinose and xylose.

3.5. Carbohydrate composition of HD-2189

Wheat variety HD-2189 was identified as poor chapati making variety, and was selected to study the nature of pentosans. The flour contained carbohydrates as major component with small amount of uronic acid (Table 5). Sugar composition of the flour showed glucose, mainly, with small amounts of arabinose, xylose and mannose along with a low quantity of rhamnose/fucose. The water-soluble polysaccharides comprised predominantly glucose (94%), which might be coming from β-glucans, resistant starch, and unhydrolysed starch molecules. Arabinose, xylose and glucose were the major sugars observed in barium hydroxide extract. A/X ratio was found to be lower (0.65) in these polysaccharides, indicating that they may be due to lower degree of branching or due to the presence of the xyloglucan (Dupont & Selvendran, 1987; O'Neill & Selvendran, 1985) and arabinan types of polysaccharides. Michniewicz, Biliaderis, and Bushuk (1990) reported that degree of

Table 4
Carbohydrate composition (%) of whole-wheat flour (MACS-2496) and its isolated fractions

Fractions	Yield	Total sugar	Uronic acid	Sugars identified (%)						
				Rha/Fuc	Ara	Xyl	Man	Gal	Glc	Ara/Xyl ratio
Flour	(100)	70.8	4.7	0.82	6.34	7.86	1.03	–	84.0	0.80
WSP	10.9	72.0	2.0	0.96	5.69	4.99	1.83	–	86.5	1.14
BE	2.9	76.0	3.0	–	41.0	51.2	–	–	7.80	0.80
Hem A	1.2	73.8	6.0	1.33	4.48	42.5	–	–	51.7	0.10
Hem B	1.4	80.0	4.0	–	21.4	47.8	–	–	30.7	0.44
AIR	4.5	65.8	4.1	–	11.0	13.1	2.66	0.31	72.9	0.83

Abbreviations as in Table 2.

Table 5
Carbohydrate composition (%) of whole-wheat flour (HD-2189) and its isolated fractions

Fractions	Yield	Total sugar	Uronic acid	Sugars identified (%)						
				Rha/Fuc	Ara	Xyl	Man	Gal	Glc	Ara/Xyl ratio
Flour	(100)	76.2	4.0	0.32	3.8	4.50	3.20	–	88.2	0.84
WSP	14.5	89.3	3.0	0.43	2.6	3.00	0.36	–	93.6	0.86
BE	5.5	85.0	1.6	–	26.7	40.7	–	1.2	31.4	0.65
Hem A	0.5	95.5	3.3	–	5.0	59.8	0.80	–	34.4	0.08
Hem B	0.8	84.0	1.9	–	30.6	24.6	2.0	0.4	42.4	1.24
AIR	3.2	72.7	2.6	–	10.0	8.10	1.90	–	80.0	1.23

Abbreviations as in Table 2.

branching (A/X ratio) ranged between 0.58 and 0.96 in some of the Canadian wheat varieties of diverse technological characteristics. Vinkx and Delcour (1996) reported A/X ratio as low as 0.21 for rye arabinoxylans. The hemicellulose A contained xylose, predominantly (60%), with a moderate amount of glucose along with a small amount of arabinose. This fraction may be a mixture of xylan type polysaccharide with small amounts of arabinoxylan type of polysaccharides. Arabinose, xylose and glucose were the major sugars found in hemicellulose B fraction. Alkali-insoluble residue contained mainly, glucose (80%), with small amounts of arabinose and xylose.

Chapati is the most popular unleavened flat bread in India and is consumed during almost every meal of the day. Softness and flexibility are the most important quality parameters of chapati. Wheat varieties, GW-322 and DWR-162 revealed good chapati making characteristics, while, MACS-2496 and HD-2189 had poor chapati making quality (Tables 1 and 6). The wheat varieties studied showed differences in the nature of constituent sugars and hence suggested variations in the nature of polysaccharides in wheat varieties having differences in chapati-making characteristics (Tables 2–6). Some of the more important chemical differences in relation to their chapati making quality are summarized in Table 6. Pentosans are the major constituent of non-starch polysaccharides in wheat. Barium hydroxide soluble polysaccharides, hemicelluloses A and B were rich in pentosans. Arabinoxylans of barium hydroxide extracted polysaccharides might be highly branched in wheat varieties, DWR-162 and GW-322 having good chapati making quality as indicated by high A/X ratio, where as those of MACS-2496 and HD-2189 wheat varieties (poor-chapati making characteristics) showed lower degree of branching as revealed by low A/X ratio (Table 6). Arabinoxylans in different varieties of wheat are known to vary and have a relationship to bread making quality. The contents of arabinose and xylose were reported to be higher in varieties of wheat that have good tandoori making quality (Saxena, Salimath, & Haridas Rao, 2000). Hemicellulose A in good chapati making varieties (DWR-162 and GW-322) is mainly xylan type with a small amount of glucan type polysaccharide (Table 6), where as in poor varieties, (MACS-2496 and HD-2189) they are mainly xyloglucan type (Dupont & Selvendran, 1987; O'Neill & Selvendran, 1985). Some amount of glucose in these fractions may originate from β-glucans (Mares & Stone, 1973; Henry, 1987) or α-glucan polymers that are entrapped within the insoluble polysachharide matrix (Smith & Hartley, 1983), or may be as a result of incomplete removal by amylase treatment. The ratio of A/X, indicative of degree of branching of arabinoxylans, may play an important role in the physico-chemical properties of these constituents and branching is known to affect confirmation of these biopolymers in solutions (Andrewartha, Phillips, & Stone, 1979).

The arabinoxylans are known to bind strongly to cellulosic polysaccharides and are also bound with ferulic acid residues forming strong ester and ether linkages (Ford & Hartley, 1989; Hartley & Jones, 1976). These associations make extractions of polysaccharides difficult. The alkali-insoluble residues (AIR) are mainly cellulosic in nature in poor varieties (MACS-2496 and HD-2189) and appear to be more tightly bound to pentosans in good chapati making varieties (DWR-162 and GW-322). Arabinoxylans play an important role in determining bread-making quality. Differences in molecular features of arabinoxylans, including degree of branching, spatial arrangement of arabinosyl residues along the xylan backbone, and the ferulic acid content, could alter the visco-elastic properties of the gels.

Table 6
Comparison of chapati making quality in wheat varieties with a few chemical characteristics

Varieties	Overall quality (50)	Ara/Xyl ratio		Xyl/Glc ratio
		Flour	BEP	Hem A
DWR-162	42.0	1.26	1.68	3.22
GW-322	41.7	1.32	1.14	4.83
MACS-2496	35.2	0.80	0.80	0.82
HD-2189	36.0	0.84	0.65	1.72

Abbreviations as in Table 2.

4. Conclusion

The results presented in this communication indicate variations in pentosans mainly along with their association with other non-starch polysaccharides in varieties differing in chapati making characteristics. More important differences in chemical characteristics linked to their chapati making quality are presented in Table 6. The pentosans were extracted mainly with barium hydroxide and these pentosans had higher A/X ratio in varieties having good chapati making quality. The hemicellulose A was mainly xylan-type in good chapati making varieties with small amounts of glucan type polysaccharides, whereas in poor chapati making varieties, they are xyloglucan type. The alkali-insoluble residue (AIR) is more cellulosic in poor chapati making varieties and appears to be more tightly bound to pentosans in good varieties. Ester linkages of pentosans with ferulic acid and other non-starch polysaccharides may be of interest and need to be investigated.

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